

Original Article

Determination of Reference Range of Glycosylated Haemoglobin (HbA1c) for Different Age Groups

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Abstract

Objective: To determine the reference interval of glycosylated haemoglobin for different age groups in our local Pakistani population.

Subjects & methods: Total number of 164 healthy adults (91 females and 73 males), were selected through non-probability consecutive sampling. After informed consent, venous blood sample (3-4 ml) from each subject was collected in EDTA vial under aseptic conditions. Haemolysed blood was used for estimation of HbA1c by Fast Ion-Exchange Resin Separation method on Microlab 300. The participants of the study were divided into three groups according to their age distribution.

All data was analysed using Microsoft Excel and statistical software Minitab15. Mean and SDs were calculated for each group. Reference range of HbA1c was calculated for each group by using the formula $\text{Mean} \pm 2\text{SD}$.

Results: One hundred and sixty-four healthy individuals were included in the study. Mean age of the individuals was 32 ± 11.6 years and mean HbA1c level was $4.17 \pm 0.93\%$. The reference range of HbA1c for age group 20-30 years was 2.09-5.57% with the mean value of $3.83 \pm 0.87\%$. The reference range of HbA1c for age group 31-40 years was 2.63-5.99% with the mean value of $4.31 \pm 0.84\%$. The reference range of HbA1c for individuals > 40 years of age was 2.8-6.4% with the mean value of $4.31 \pm 0.9\%$.

Conclusion: For proper assessment of the diabetic status, it is mandatory that every lab should establish its own reference range of HbA1c for both genders and different age groups representing its patient population.

Key words: Glycosylated hemoglobin ,Pakistan, Population, Reference range

Introduction

Diabetes Mellitus is a major healthcare issue worldwide and the global burden of diabetes has been rising in both developed and developing countries. Type 2 diabetes accounts for majority (90%) of the cases of diabetes mellitus. Sedentary lifestyle and eating habits are the major contributory factors. According to National Diabetes Survey, 14.5 million cases of type 2 diabetes will be there in Pakistan till 2025. Regarding prevalence of Diabetes, among the top ten countries of the world, Pakistan will be at fourth number in 2025.¹ Diabetes Mellitus is associated with long term microvascular and macrovascular complications like retinopathy, neuropathy, and nephropathy thus increasing the overall morbidity and mortality.^{2,3}

Glycosylated haemoglobin(HbA1c) is an index of glycemic status in diabetics mellitus. Blood glucose attaches HbA1c non-enzymatically to N-terminal valine residue of the β -chain of hemoglobin resulting in the formation of glycated hemoglobin.⁴ It provides an estimate of diabetic control over the preceding two to three months.^{5,6} In addition to monitoring of diabetic control, American Diabetic Association in 2010, finally recommended HbA1c to be used as a diagnostic test.⁷ After a lot of resistance, WHO also endorsed this recommendation in 2011 and HbA1c is now widely used as a diagnostic test.³ The biggest advantage of HbA1c is that fasting state is not required. This has led to broadening of screening base and many of those patients can be screened who cannot get their test done in fasting state. In addition to that, HbA1c analysis is associated with less biological variability and pre-analytic instability than that of fasting plasma glucose.⁸⁻¹¹ It is not influenced by acute changes in blood glucose levels that occur in stress or illness. So the routine analysis of HbA1c has gained importance regarding diagnosis, management and prevention of long term complications of diabetes.⁷

Irrespective of all advantages of HbA1c, there are some challenges related to its analysis. The foremost is the lack of standardization of methods used for HbA1c analysis by most of the laboratories. There are different types of methods used for estimation of HbA1c level.^{12,13} They include High

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performance liquid chromatography, affinity chromatography, Cation exchange chromatography, isoelectric focusing, radioimmunoassay, spectrophotometric assay, electrophoresis and electrospray ionization mass spectrometry.¹³ Point of care testing devices have also been introduced but they are not much accurate and precise.⁷ Although these assays are based on different principles, standardization should be achieved according to the reference method of the International Federation of Clinical Chemistry (IFCC) and the National Glycohemoglobin Standardization Programme (NGSP).^{14,5} There is broad variation in method selectivity by variable laboratories in the world leading to inconsistent results. Harmonization of the results is the need of the day to achieve best clinical utility of HbA1c. Global standardization of HbA1c results with respect to post-analytical issues like measurement units and reference interval should also be addressed.¹⁵ Reference values of HbA1c in different populations based on different age groups, ethnicity and specific patient group remains a challenge. It is mandatory that every lab should establish its own reference range.^{4,12} We planned the study to achieve the reference interval of glycosylated haemoglobin for different age groups of Pakistani population in Wah Cantt.

Subjects and Methods

This cross sectional study was carried out in the Department of Pathology at Wah Medical College, Wah Cantt from February to August, 2016. Total number of 164 healthy adults (91 females and 73 males), comprising the medical students and staff members of Wah Medical College/hospital who were normoglycemic and normotensive, had no other co-morbid condition were selected through non-probability consecutive sampling while the subjects who were diabetic or had impaired glucose tolerance were excluded based on history and laboratory investigations. After informed consent, venous blood sample (3-4 ml) from each subject was collected in EDTA vial under aseptic conditions. Hemolysed blood was estimated for HbA1c within one hour after sample collection on Microlab 300, using Human kit based on Ion-Exchange Chromatography technique.

The participants of the study were divided into three groups according to their age distribution. Group 1 comprised of 75 individuals having age range of 20-30 years. Group 2 comprised 46 individuals having age range of 31-40 years. Group 3 had 43 participants more than 40 years of age.

All data was entered and analyzed using Microsoft Excel and statistical software Minitab15. Mean and SDs were calculated for each group. Reference range of HbA1c was calculated for each group by using the formula $\text{Mean} \pm 2\text{SD}$.

Results

One hundred and sixty four healthy individuals were included in the study. Mean age of the individuals was 32 ± 11.6 years and mean HbA1c level was $4.17 \pm 0.93\%$.

Male and female distribution of the subjects is shown in Figure 1. Mean age of all the three groups were 22 ± 2.93 , 35 ± 3.2 and 47 ± 8.4 years respectively. The reference range of HbA1c for age group 20-30 years was 2.09-5.57% with the mean value of $3.83 \pm 0.87\%$.

The reference range of HbA1c for age group 31-40 years was 2.63-5.99% with the mean value of $4.31 \pm 0.84\%$.

The reference range of HbA1c for individuals >40 years of age was 2.8-6.4% with the mean value of $4.31 \pm 0.9\%$.

Mean values of HbA1c for each group along with their standard deviations are shown in Figure 2. The reference range of HbA1c for females (n=91) was 2.51-6.11% with the mean value of $4.31 \pm 0.9\%$. In males (n=73) reference range of HbA1c was obtained as 2.11-5.87% with the mean value of $3.99 \pm 0.94\%$.

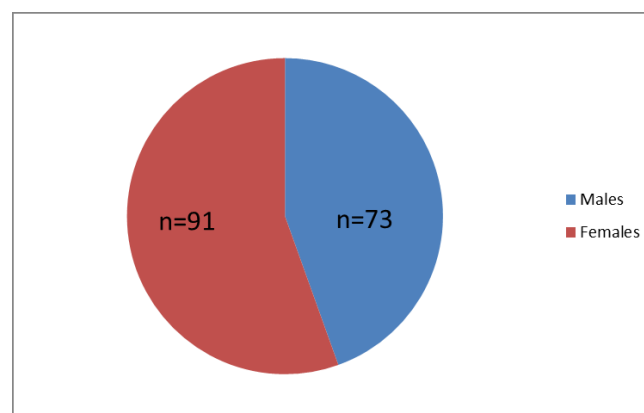


Figure 1: Male and female distribution of subjects

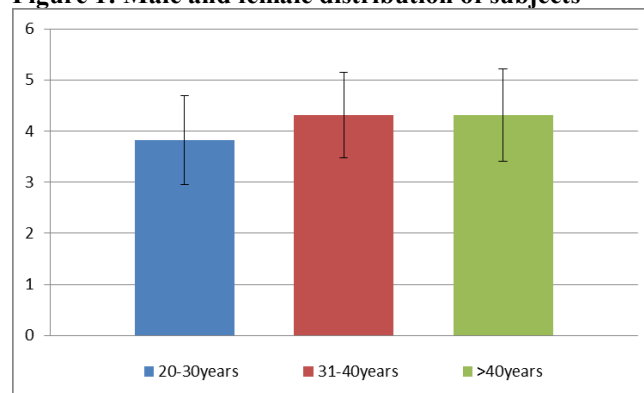


Figure 2: Mean and Standard deviations of HbA1c for individual groups

Discussion

In our study we have established the reference interval of glycosylated haemoglobin for our local population. A study was conducted at Armed Forces institute of Pathology, Rawalpindi, Pakistan to determine the reference ranges of HbA1c in 254 healthy adults (18-80 years) included 169 males and 85 females. In total population reference range of HbA1c was 4.6–6.56%. In our study mean value of HbA1c in total population was $4.17 \pm 0.93\%$. When analysis was

done according to gender at AFIP, no significant difference was found between males and females. In our study reference range of HbA1c for females was 2.51-6.11% while in males it was 2.11-5.87%. Study conducted at AFIP showed increased HbA1c levels in healthy elderly population. In healthy persons of less than 40 years of age, reference range of HbA1c was 4.52-6.4% while in individuals having age more than 40 years; it was 4.8-6.68%. These results are comparable to our study. We also found high reference range of HbA1c in elderly.¹⁶

The difference of values among different populations may be due to diversity in nutritional habits and life styles. Reference range values determined for Iraqi population for subjects age ranged between 20-75 years old was 5.34±0.67 for females and 5.67±0.73 for males.¹² The values are slightly higher than our results, which are 4.31±0.9 and 3.99±0.94 for females and males respectively. In addition to that, the mean value of HbA1c in females is slightly higher than that of males, which is in contradictory to Iraqi population.

In Ghanaian population, the mean value of HbA1c (for both sexes) was 5.4 ± 0.84% and reference range for the glycosylated haemoglobin was 3.72-7.08%, which is again different from that of Pakistani population.⁴ In contrast to our findings, the reference range values of our neighbouring Indian population (30-70 years) were found to be slightly higher. In Indian women, mean HbA1c value was 6.5±0.74% and overall range was found to be 4.8-7.56%. On the other hand, men had mean HbA1c levels of 6.27±0.94% and overall range was 4.2-7.56%.²

As different populations have different HbA1c levels and there is diversity in methods of estimation, it is recommended that every laboratory should establish its own reference interval of HbA1c for its own population

Conclusion

For proper assessment of the diabetic status, it is mandatory that every lab should establish its own reference range of HbA1c for both genders and different age groups representing its patient population.

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Conflict of Interest

This study has no conflict of interest as declared by any author.

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Authorship Contribution:

Author 1,2: Conception, Synthesis and Planning of the research, Active participation in active methodology, Interpretation, analysis and discussion

Author 3: Conception, Synthesis and Planning of the research, Interpretation, analysis and discussion